URINARY INDICATORS OF STRESS:

Effects of Exposure to Simulated Sonar Noise for 8 to 23 Days

by

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SUMMARY PAGE

THE PROBLEM

To estimate levels of biological stress through the integration of biochemical information obtained from a variety of analyses performed on urine samples collected from subjects experiencing the environmental hazards of exposure to distracting noise and extended confinement.

FINDINGS

Significantly high correlations among excretion rates of various urinary steroids and nitrogenous metabolites, electrolytes and osmolarity have been established. Estimating equations have been obtained for deducing approximate levels of steroids from several combinations of measurements made for simple metabolites. The most efficient group of indicator components has been creatine, urea nitrogen, osmolality and potassium.

APPLICATION

The technique described may be utilized to provide prompt approximations of stress since methods are available for rapid analyses of the metabolic products measured in this study. Expeditious evaluation of stress for divers or other personnel exposed to unusual or hazardous circumstances can prove essential to the successful performance of critical operations or even the saving of life.

ADMINISTRATIVE INFORMATION

This investigation was conducted as part of Bureau of Medicine and Surgery Research Unit MR041.06.01-0026BXKK. The present report is Number one on this work unit. It was submitted for review on 7 August 1973, approved for publication on 15 October 1973, and designated as NavSubMedRschLab Report No. 766.

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ABSTRACT

Studies have been made of the relationships among excretion rates of urinary steroid hormones (indicators of stress) and a variety of other urinary metabolites in healthy young men subjected to mild laboratory-controlled stress consisting of simulated sonar noise and continuous confinement for 8 to 23 days. The objective of the study was to evaluate the stress of an increased noise component in the environment of Naval personnel living on submarines. An approach to the problem has been to utilize orderly means for evaluation of the several kinds of urinary data commonly obtained for stress analysis. Significant correlations among the excreted hormones and accompanying metabolic components make possible an estimation of steroids, and thus of stress from any of several combinations of metabolic data. Using the techniques proposed, it is possible to evaluate stress responses from information more readily available than from the more complex and protracted steroid measurements. It is anticipated that these techniques might be applied to the estimation of stress in operational situations in which human or material damage might be averted by prompt evaluation of the degree of accumulated personnel stress.

URINARY INDICATORS OF STRESS:

I. Effects of Exposure to Simulated Sonar Noise for 8 to 23 Days

INTRODUCTION

With health, happiness, life and property dependent upon appropriate responses in times of stress, it is highly significant for both civilian and military applications that methods be established for objectively evaluating stress in individuals and in large population groups. The correlation studies reported were undertaken when it was observed that for healthy young men subjected to mild distracting noise stress combined with 1 to 3 weeks confinement under laboratory conditions, an apparent relationship exists between the excretion of 17-ketosteroids and several other urinary components. The experiments described concern one aspect of a broader investigation of some of the stresses of life aboard operational submarines.[19,23,24]

Customarily various hormone levels in the blood or urine have been utilized for the assessment of stress levels. To our knowledge these methods have not been surpassed in reliability. However, unless extremely sophisticated and specialized equipment is available[3], extensive manual manipulation of each sample is required to effect extractions or other purification procedures even if conventional automated analytical techniques are employed when applicable[6,25].

In addition to measurement of hormone levels, however, it also seems generally accepted that stress should be evaluated on the basis of several metabolic components or products[8,9,15,16,18,20]. Although we have made no attempt to quantitate stress itself, methods such as those employed here appear useful as approaches to attain some overall evaluation of stressful responses.

In a laboratory dedicated to the study of practical working situations, it is quite obvious that it is advantageous to use urine analyses for routine or repeated determination of stress-related parameters. Subjects can obviously supply urine samples with a minimum of cooperation and with little added effort. Since many of our working subjects are divers who are not notably tolerant of experimental procedures, it is a decided advantage to be able to ask for urine as opposed to blood samples.

It is our intent to present several significant correlations observed among urinary constituents which may serve as points of reference for additional studies involving evaluation of stress.

METHODS

In the experiments from which data are presented, urine samples were collected from two groups of volunteer subjects. These men were exposed to the stresses of confinement to a laboratory environment for periods of 8 days to 23 days, and to an intermittent noise

simulating that of a sonar system being developed for use on submarines. Earlier reports or reports in preparation by other investigators give the details of the noise presentation, the experimental arrangement, and the biomedical and psychophysiological evaluations of the stress situations [19,23,24]. While the subjects received balanced meals prepared at the Navy mess hall, supplementation from outside sources was permitted. Therefore, diets which are fairly typical for well nourished, young American men were received.

Urines were collected in 24 hour units in the presence of HC1; volumes were recorded and aliquots were frozen until analyzed. Urea N (UN), creatinine (Cr) and uric acid (UA) determinations were performed by standard autoanalyzer* techniques (N-13b and N-38a). 17-ketosteroids (KS) were analyzed by the method of Zak, Epstein and Kraushaar [25] which employes an autoanalyzer analysis after preliminary extraction and purification of urine samples. Osmolalities (Os) were determined using a Fiske model G-62 osmometer**, and Na+ and K+ in an IL Model 343 flame photometer#.

Ketogenic steroids were determined by measurement as Zimmerman chromagens [26] after oxidation by sodium bismuthate [2,4,17,22]. Proportions used for the oxidation step in this work, were 7 ml urine, and 7 ml glacial acetic acid plus .13±.02 gm. sodium bismuthate. After vigorous shaking, the mixture was heated for 10 min. at 56°. The tubes were then centrifuged or their contents allowed to settle and two 5 ml. aliquots of the supernate removed for duplicate analyses according to the procedure for ketosteroids.

* *Technicon Corporation, Tarrytown, New York*
** *Fiske Associates, Inc., Uxbridge, Mass.*
\# *Instrumentation Laboratory, Inc., Lexington, Mass.*

RESULTS

Since the primary interest of the current discussion concerns relationships among several urinary components, all the results of our first experiment involving a sizable number of urine samples collected for stress analysis are grouped together in Table 1. In this study, urine from 12 men was collected during an 8-day experiment. Although a trend toward slightly lower levels of ketosteroid excretion was noted during the midportion of the experimental period, all data were sufficiently similar that treatment of the entire group as a unit seemed justified. It may be seen that our impression of a relationship between excretion of ketosteroids and several of the other components analyzed is adequately supported by the data. A high degree of correlation, in fact, exists among all of the urinary parameters analyzed with the notable exception of the lesser correlations with uric acid. All of the r values of Table I indicate highly significant correlations, $p<.01$, with the exception of the uric acid-urea nitrogen combination. Since creatinine, urea nitrogen, and osmolality correlate highly with the ketosteroids, it is to be expected that they should correlate highly with each other.

The data of Table II (N-182) were obtained from an experiment, enlarging

Table I. Correlation Among Urinary Components For 96 Man-Days of Mild Stress

	24 Hr. Total*	17KS†	Cr	UA	UN	Os
17-KS	15.2 ±.7					
CREATININE	1.67±.05	.76				
URIC ACID	.30±.05	.42	.36			
UREA N	11.6 ±1.2	.75	.83	.18		
OSMOLES	1.09±.09	.77	.84	.37	.94	
VOLUME	1.28±.13	.63	.56	.49	.63	.75

* Mg (for 17 KS) grams, osmoles or liters excretion per 24 hours
† Coefficients of correlations: 17KS vs Cr, UA, UN, Os

upon the results of the preceding study, in which urines were collected from a group of 9 or 10 men over a 23 day experimental period. In this series Na+ and K+ concentrations were determined in addition to the parameters of Table I. A compilation of the two sets of data, also shown in Table II (N=278), provides the most extensive set of correlations for studying interrelations between ketosteroids and the other urinary components which we have yet available.

Because it is very much easier to obtain any or all of the other data listed in Tables I and II than those for ketosteroids, it was deemed worthwhile to study the possibility of employing these more readily available data for making predictions of ketosteroid values. Tabulated in the footnotes of Table II are the estimating equations and coefficients of multiple correlation for each of the four data sets included in the table.

To preliminarily study the usefulness of ketogenic steroid or hydroxysteroid determinations, the ketogenic procedure was run on urines from two days representing the middle of the second experimental period. Hydroxysteroids may be estimated as the difference between ketogenic steroid and ketosteroid values[1,17,22]. Excretion data for the various urinary metabolites including the additional steroid hormone groups are shown in Table III. In addition to the total daily excretions,

Table II. Correlation of Urinary Constituents With Ketosteroids

	N = 182			N = 278		
	24 HR. EXCRETION	CORRELATION WITH 17-KS		24 HR. EXCRETION	CORRELATION WITH 17-KS	
		PER 24 HR.[1]	PER VOL[2]		PER 24 HR.[3]	PER VOL[4]
VOLUME*	1.77±.05	.34		1.59±.04	.44	
CREATININE	1.74±.03	.34	.65	1.72±.03	.44	.60
URIC ACID	.76±.02	.27	.28	.60±.02	.36	.17
UREA N	13.3 ±.3	.28	.51	12.6 ±.3	.41	.53
OSMOLES	1.04±.02	.37	.56	1.05±.02	.44	.50
NA	.173±.005	.36	.37			
K	.086±.002	.38	.52			
17-KS	19.4 ±.8	-	-	17.98±.55		

	r	Estimating Equation
[1]	.44	$-.507 + 4.429\,Cr - .286UN + 9.005\,O_s + 77.63\,K$
[2]	.66	$.422 + 8.362\,Cr - .349UN + 4.197\,O_s + 46.89\,K$
[3]	.53	$-1.202 + 4.749\,Cr - .045UN + 7.670\,O_s + 5.957\,UA$
[4]	.62	$1.797 + 6.065\,Cr + .318UN + 1.513\,O_s + 3.610\,UA$

* See table I for units (equivalents for Na and K).

Table III. Urinary Excretion and Body Weight Data For Exploratory Ketogenic Steroid Excretion Study*

	Excretion per 24 hours	Conc. per Liter	Excretion per gm. Cr
VOL	1.88 ±.21		
UA	.80 ±.07	.45 ±.02	.45 ±.04
Cr	1.84 ±.14	1.16 ±.13	
UN	14.1 ±1.3	8.6 ±.9	7.6 ±.4
Na	.173±.023	.098±.008	.094±.007
K	.103±.012	.059±.004	.055±.003
Os	1.01 ±.05	.62 ±.05	.60 ±.06
KS	16.3 ±1.3	10.4 ±1.6	9.5 ±.8
KGS	44.5 ±4.7	25.3 ±1.7	25.2 ±2.3
OHS	28.2 ±3.9	14.9 ±.8	15.7 ±1.8
Na/K	1.69 ±.10		
Wt	80.9 ±1.8		

* For 18 man-days as described in text. Liters, grams, osmoles, mg (for steroids) equivalents (for Na and K) or Kg (for body wt).

comparative values for the excretion rates based on concentration per liter and concentration per gm. of creatinine are also illustrated. In Table IV correlations are made among the various urinary components and the steroid values, as well as correlations with the body weights of the subjects.

Table IV. Correlation of Steroid Excretion and Body Weight With Several Urinary Components

	A. Daily Correlation			B. Correlation per Volume			C. Correlations per Gm Cr			BW Corr.
	KS	KGS	OHS	KS	KGS	OHS	KS	KGS	OHS	
UA	.46	$.79^{3}$	$.80^{3}$	.41	.48	.19	.40	$.72^{2}$	$.73^{2}$	.02
Cr	.27	$.47^{1}$	$.48^{1}$	$.73^{2}$	$.72^{2}$	.03				-.25
UN	.23	$.47^{1}$	$.49^{1}$	$.59^{1}$	$.54^{1}$	-.08	-.12	-.02	.04	-.18
Na	.30	$.68^{2}$	$.72^{2}$	.24	.28	.11	.13	.38	.42	-.16
K	$.54^{1}$	$.82^{3}$	$.81^{3}$	$.66^{2}$	$.65^{2}$	.03	.37	$.67^{2}$	$.68^{2}$	-.14
Os	.23	$.55^{1}$	$.59^{1}$	$.66^{2}$	$.58^{1}$	-.12	$.64^{2}$	$.65^{2}$	$.54^{1}$	.23
Mean	.34	.63	.65	.55	.54	.03	.28	.48	.48	-.08
Na/K	$-.51^{1}$	-.21	-.09	-.34	-.33	-.01	-.25	-.14	-.07	
Wt	$.56^{1}$	.20	.05	.48	.42	-.10	$.65^{2}$	.43	.26	
Vol	$.50^{1}$	$.89^{3}$	$.91^{3}$	$.57^{1}$	$.50^{1}$	.10	.07	$.58^{1}$	$.70^{2}$	
Ster.*	$a=.70^{2}$	$b=.52^{1}$	$c=.97^{3}$	$a=.89^{3}$	b=-.17	c=.30	$a=.70^{2}$	b=.44	$c=.95^{3}$	

*Steroids: a = KS vs KGS, b = KS vs OHS, c= KGS vs OHS

$^{1}p<.05$, $^{2}p<.01$, $^{3}p<.001$

DISCUSSION

Although the correlations among the steroid excretion data and the other urinary components are somewhat different for the first and second experiments, the fact is adequately substantiated that significant correlations between these data do exist. It may be speculated that part of the difference between the two sets of data is related to the differences in the two groups of subjects. In the first experiment all of the subjects were enlisted Naval personnel whereas in the second experiment considerable variability in such factors as occupations, routines, and dietary habits was introduced with the employment of a mixed Naval and civilian group. All of the men were in an age range comparable to that found in the submarine service. Despite apparent differences between the two groups of data, however, the similarities between corresponding parameters in the sets indicate that the differences are quantitative rather than qualitative in nature.

It is significant to note that the overall observation from the two experiments, as summarized in Table II, is that equal or better correlations with 17-ketosteroid excretion is obtained when the calculations are performed on the basis of unit concentrations (i.e., mg/1, eq/1, etc.) than on the basis of total excretion during a 24 hour period. While it is recognized that our concentration data are based on collections obtained over 24 hours, these represent average values which should be independent of daily cycles. For our purposes it seems important to utilize such "average" values for calculations of estimating equations which might be employed with data obtained from samples collected during any period of the day. Under practical conditions of collecting samples from human subjects who are undergoing work stresses, urine samples representing almost any period of the 24 hour day might be available.

In Table IV, the relatively high correlations of volume, urea nitrogen, creatinine, potassium or osmolality to the various steroid hormones, compared either on the basis of 24-hour excretions or per volume of urine, corroborate the high individual and multiple correlations obtained with these variables against ketosteroids in Tables I and II. It is also of interest that, whereas the correlations of ketosteroids with metabolite/Cr ratios or correlations with 24 hour excretions are generally lower than correlations per unit volume (Table IV), the corresponding relationships to hydroxysteroids tend to be closer under the former two circumstances. The correlations of uric acid to KGS or OHS tend to be higher per 24 hour excretion and per gm. of creatinine than per volume of urine. Sodium, on the other hand, has statistically significant correlations with OHS and KGS on a 24 hour basis but lower correlations per unit volume. As might be predicted, urine volume correlates low or negatively with steroid output per liter while the strong relationship of total excretion to volume seen earlier is still evident.

Pertinent observations may also be made concerning the interrelations

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13. ABSTRACT

Studies have been made of the relationships among excretion rates of urinary steroids hormones (indicators of stress and a variety of other urinary metabolites in healthy young men subjected to mild laboratory-controlled stress of simulated sonar noise and continuous confinement for 8 to 23 days. The objective of the study was to evaluate the stress of an increased noise component in the environment of Naval personnel living on submarines. An approach to the problem has been to utilize orderly means for evaluation of the several kinds of urinary data commonly obtained for stress analysis. Significant correlations among the excreted hormones and accompanying metabolic components make possible an estimation of steroids, and thus of stress from any of several combinations of metabolic data. Using the techniques proposed, it is possible to evaluate stress responses from information more readily available than from the more complex and protracted steroid measurements. It is anticipated that these techniques might be applied to the estimation of stress in operational situations in which human or material damage might be averted by prompt evaluation of the degree of accumulated personnel stress.

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